Attorney Docket No. 5849.200-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Berka *et al.* Confirmation No. 6980

Serial No.: 09/533,559 Group Art Unit: 1631

Filed: March 22, 2000 Examiner: J.S. Brusca

For: Methods For Monitoring Multiple Gene Expression

AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

This is a response to the Advisory Action dated May 22, 2003. Claims 103-110 are pending in the present application. It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the following remarks is requested.

I. The Rejection of Claims 103-110 under 35 U.S.C. § 101

Claims 103-110 stand rejected under 35 U.S.C. § 101 on the ground that the claimed invention lacks patentable utility for reasons of record. The Advisory Action states:

> The applicants state that example 16 on pages 357-358 demonstrates how to use the claimed invention. Example 16 refers to measurement of polynucleotide sample hybridization to a microarray comprising EST probes of Fusarium venenatum, while the claims are drawn to use of a microarray comprising EST probes of Aspergillus oryzae. The applicants state that measuring the ability of a polynucleotide sample to hybridize to the probes on a microarray of Aspergillus oryzae allows for measurement of expression profiles in the sample, and further allows comparison of samples prepared from cells grown under different conditions to determine the effect of the condition on the expression profile of the EST sequences. Because the function of the EST-linked genes has not been clearly established, and because it is not known what differences of expression would be seen if the claimed method were performed, there would be further experimentation required on the claimed method to establish whether useful information would be produced by the claimed method. This is not consistent with a substantial patentable utility.

This rejection is respectfully traversed.

Applicant has asserted in previous Amendments of August 23, 2002 and May 7, 2003, that one of ordinary skill in the art using Applicants' disclosure would be able to set-up and use the methods of the present invention to monitor global expression of a plurality of genes from a filamentous fungal cell with respect to a particular phenotype.

Dr. Randy Berka has prepared a Declaration (see attached) in support of Applicant's arguments.

Dr. Berka disagrees with the Office Action's contention that the "claimed combination of nucleic acids is not supported by a substantial utility" because "[n]o evidence has been disclosed that the elected SEQ ID NOS allow for determination of the state or type of cell that is assayed by the claimed method of using an array of *Aspergillus oryzae* ESTs." He describes the use of an array of *Aspergillus oryzae* ESTs to study a recombinant *Aspergillus oryzae* strain containing a *Thermomyces lanuginosus* lipase gene (Le-1) and a yield- and morphologically improved mutant (7-1) of Le-1 to understand why Le-1 displays a very pronounced "ballooning" phenotype when grown in laboratory fermentors and after approximately 90 hours of fermentation production of the lipase ceases, while mutant 7-1 displays a much lower degree of ballooning and does not stop producing lipase after 90 hours.

Dr. Berka indicates that analysis of the microarrays revealed a number of genes whose transcript levels differed between Le-1 and 7-1. Samples of mRNA extracted from strains Le-1 and 7-1 cultivated in 1 L fermentors for 3 or 4 days were labeled and hybridized to a microarray containing the *Aspergillus oryzae* ESTs described in Appendix A. Among these genes were 53 ribosomal genes and 27 genes identified as being involved in cell wall synthesis and morphogenesis, indicating that general protein production and the cell wall metabolism is regulated differently in the strains, which fit well with the observation that the morphology of the two strains is different, especially with regard to the degree of "ballooning" when expressing the lipase gene.

Dr. Berka indicates that the 53 ribosomal genes are consistently down-regulated in 7-1 compared to Le-1 on both day 3 and day 4 and that the consistent down-regulation of genes involved in translation indicates that 7-1 has generally reduced translational activity compared to Le-1, which may relieve some of the secretional stress and reduce the degree of ballooning seen in Le-1. He also suggests that the down-regulation may reduce the counter-selective pressure against lipase-producing cells that may be responsible for the rapid loss of expression potential in Le-1 cultures after 90 hours of fermentation, allowing 7-1 to retain its expression potential throughout the fermentation.

Dr. Berka also discloses that 27 genes involved in cell-wall synthesis are differentially regulated where twenty of these genes are consistently up-regulated while seven are down-

regulated in 7-1 compared to Le-1. The results suggest that the β-glucan synthesis pathway is up-regulated in strain 7-1 compared to Le-1. The physiological effect of the increased activity of this pathway is consistent with the reduced ballooning seen in 7-1, since it may be that the ballooning phenotype could be caused by a deficiency of cell-wall components at the hyphae elongation sites, and that this deficiency could be an effect of immense overloading of the secretion machinery in the cells. While the up-regulation of proteins that take part in cell-wall synthesis *in situ* does not reduce the pressure on the secretion pathway, it may increase the secretion of cell-wall synthesis enzymes at the expense of other, less crucial, enzymes.

Dr. Berka concludes that the results clearly demonstrated that the microarrays produced from the *Aspergillus oryzae* ESTs provide a powerful tool to study the effect of strain differences on global gene expression in the cells, especially in the context of mutants produced by "classical" means (*i.e.*, by radiation or chemical mutagenesis). He further concludes that without this technique it would be an almost impossible task to identify the pathways and ideally the genes that are affected by the introduced mutations.

Applicants assert, therefore, that the claimed method using the combination of nucleic acids of *Aspergillus oryzae* ESTs is supported by a substantial patentable utility. One of ordinary skill in the art using Applicants' disclosure would be able to set-up and use the methods of the present invention to monitor global expression of a plurality of genes from a filamentous fungal cell with respect to a particular phenotype.

For the foregoing reasons, Applicants submit that the rejection under 35 U.S.C. § 101 has been overcome and respectfully request reconsideration and withdrawal of the rejection.

II. The Rejection of Claims 103-110 under 35 U.S.C. § 112, First Paragraph

Claims 103-110 stand rejected under 35 U.S.C. § 112, first paragraph, on the ground that one skilled in the art would not know how to use the claimed invention since it is not supported by a substantial utility or a well established utility, as described in Section I. This rejection is respectfully traversed.

Based on Applicants' arguments in Section I, Applicants assert that one skilled in the art would know how to use the claimed invention because it is supported by a substantial utility.

For the foregoing reason, Applicants submit that the rejection under 35 U.S.C. § 112 has been overcome and respectfully request reconsideration and withdrawal of the rejection.

III. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the

undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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